

**The Efficacy of Symbion™ In The Treatment Of Irritable Bowel
Syndrome: A Randomized, Double-Blind Placebo Controlled
Clinical Trial
Protocol number: 01-PBI-2012**

A Study Sponsored by Pharmabiotix Inc

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**A Study Conducted By The Northern Alberta Clinical Trials And Research
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BACKGROUND AND RATIONALE:

Irritable Bowel Syndrome (IBS) is a functional illness, which is characterized by abdominal pain or discomfort associated with change in bowel habits and characteristics of disrupted defecation (1). IBS is one of the most common gastrointestinal (GI) disorders with one tenth to one fifth of the general adult population, with female predominance, reporting IBS symptoms (1).

IBS is associated with a huge financial burden represented by direct medical cost and loss of productivity associated absenteeism of work (2), as well as deterioration of the individual's quality of life both socially and professionally (3).

Nearly 15% of the North American population is suffering from IBS representing one of the highest rates in the world (4). One of the major problems associated with IBS is that there is no approved medication to treat IBS, the treatment that the IBS patient receives aims to address his/her concerns and relief his/her symptoms (5).

What Are Probiotics?

Probiotics are living microscopic organisms, or microorganisms, that scientific research has shown to benefit your health. Most often they are bacteria, but they may also be other organisms such as yeasts. In some cases they are similar, or the same, as the "good" bacteria already in your body, particularly those in your gut.

The most common probiotic bacteria come from two groups, *Lactobacillus* or *Bifidobacterium*, although it is important to remember that there are many other types of bacteria that are also classified as probiotics (eg, *Streptococcus*, *E. coli*). Each group of bacteria has different species, and each species has different strains. This is important to remember because different strains have different benefits for different parts of your body. For example, *Lactobacillus casei* Shirota has been shown to support the immune system and to help food move through the gut, but *Lactobacillus bulgaricus* may help relieve symptoms of lactose intolerance, a condition in which people can't digest the lactose found in most milk and dairy products. In general, not all probiotics are the same. They don't all work the same way.

Scientists are still sorting out exactly how probiotics work, but there are a few possibilities:

- They may stick to the gut wall and prevent harmful bacteria from attaching there and growing.
- They may boost your immune system by producing antibodies for certain viruses.
- They may produce substances that prevent infection.
- They may send signals to your cells to strengthen the mucus in your intestine and help it act as a barrier against infection.

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- They may inhibit or destroy toxins released by certain “bad” bacteria that can make you sick.
- They may produce B vitamins necessary for metabolizing the food you eat, warding off anemia caused by deficiencies in B-6 and B-12, and maintaining healthy skin and a healthy nervous system.

Common Uses For Probiotics

Probiotics are most often used to promote digestive health. Because there are different kinds of probiotics, it is important to find the right one for the specific health benefit you seek. Researchers are still studying which probiotic should be used for which health or disease state. Nevertheless, probiotics have been shown to help regulate the movement of food through the intestine. They also may help treat digestive disease, something of much interest to gastroenterology scientists. Some of the most common uses for probiotics include the treatment of the following:

Irritable Bowel Syndrome (IBS). IBS is a disorder of movement in the gut. People who have IBS may have diarrhea, constipation, or alternating bouts of both. IBS is not caused by injury or illness. Often the only way doctors can diagnose it is to rule out other conditions through testing.

Probiotics, particularly *Bifidobacterium infantis*, *Sacchchromyces boulardii*, *Lactobacillus plantarum* and combination probiotics may help regulate how often people with IBS have bowel movements. Probiotics may also help relieve bloating from gas. Research is continuing to determine which probiotics are best to treat IBS.

Symbion

Symbion™ is a probiotic which is composed of the following ingredients contained in a veggie capsule, being one dose:

Bacillus coagulans	200 million colony forming units	33.34mg
Bacillus subtilis	100 million colony forming units	16.67mg
Enterococcus faecium	100 million colony forming units	16.67mg
Fructo-oligosacharride	a nutrient for the packaged product	600.0mg
Total		666.68 mg

Bacillus coagulans is a non-pathogenic, Gram positive, spore forming bacteria that produces lactic acid. Though not normally found in the gut. Bacillus coagulans strains have been used as general nutritional supplements and agents to control constipation and diarrhea in humans and animals.

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Bacillus subtilis is a Gram-positive, endospore-forming soil bacteria comprising aerobic and a few facultatively anaerobic rod shaped bacteria. Bacillus subtilis was historically used to treat dysentery.

Enterococcus faecium is a facultative (growing with or without oxygen) anaerobic, Gram positive cocci that produces lactic acid. The Enterococcus faecium strain is a natural inhabitant of the mammalian GI tract.

Historically Lactobacilli and Bifidobacteria associated with food have been considered safe. Many probiotic bacteria are normal commensals of the mammalian gut flora and their established safe use over decades in a diversity of foods and dietary supplement products worldwide support this safety conclusion.

Many IBS patients who used Symbion™ as a dietary supplement to help rebalancing their gastrointestinal flora reported resolution of their IBS symptoms. The large number of positive anecdotal reports to Pharmabiotix Inc. suggest that Symbion™ may be of benefit if made into a medicinal drug to treat IBS (6). Therefore there is a need for further investigation to confirm these findings.

STUDY OBJECTIVES:

Primary objective:

To compare the effect of Symbion™ (PXO612) to placebo, administered over 12 weeks, in IBS-Diarrhea patients; on bowel movements using patient's reported stool frequency (number of stools)

Secondary Objectives:

To compare the effect of Symbion™ (PXO612) to placebo; using the patient's rating for IBS symptoms based on the assessment of the overall symptoms relief (calculated at each visit by summing the following reported symptoms scores):

- Stool frequency (number of stools/day)
- Abdominal pain (on a scale of 0-4)
- Stool consistency (on a scale of 1-7)
- Straining (yes (1) or no (0))
- Urgency (yes (1) or no (0))
- Feeling of incomplete defecation (yes (1) or no (0))
- Bloating (yes (1) or no (0))
- Passage of mucus (yes (1) or no (0))

To evaluate

- Changes in abdominal pain/discomfort, stool consistency, stool frequency, straining or urgency, feeling of incomplete defecation, passage of mucus and bloating or feeling of abdominal distention over the study period

- Changes in upper GI symptoms (i.e. heartburn, early satiety, postprandial fullness, sensation of prolonged digestion, nausea and vomiting) over the study period
- Changes in the patient's assessment of their quality of life using SF-36
- Sub group analysis regarding: gender

METHODS:

Design:

Randomized double-blind placebo controlled study

Setting:

The Northern Alberta Clinical Trials and Research Centre (NACTRC), Edmonton, Alberta, Canada

Participants:

Inclusion criteria:

- Male or female
- 18 - 65 years old
- Signed informed consent
- Mild to moderate (using Functional Bowel Disorder Severity Index (FBDSI)) IBS-Diarrhea patient:
 - IBS definition will be based on Rome criteria;
The symptoms of IBS must persist for at least 3 months and must include:
 1. Abdominal pain or discomfort which is relieved by defecation, and/or associated with a change in frequency of stool and/or consistency of stool
 2. At least two of the following, at least a quarter of occasions or days (25%):
 - A. Altered stool frequency (≥ 3 bowel movements/day or < 3 bowel movements/week)
 - B. Altered stool form (lumpy/hard or loose/watery stools)
 - C. Altered stool passage (straining, urgency or feeling of incomplete evacuation)
 - D. Passage of mucus
 - E. Bloating or feeling of abdominal distention
 - Diarrhea is defined as having loose watery stools at least three times per day

Exclusion criteria:

The patient will be excluded from the study if:

- Assessment by the treating investigator showed an evidence for cardiovascular, respiratory, urogenital, gastrointestinal/hepatic, hematologic/immunologic, head, ears, eyes, nose and throat, dermatologic/psychiatric, allergy, major surgery or other diseases as revealed by history, physical examination and existing laboratory assessments which may interfere with the administration or assessment of study medication. This should be confirmed by a pre-study medical examination performed 2 weeks prior the study.
- Pregnant or lactating
- Females at child bearing age will be excluded unless they are using acceptable birth control measures (i.e. implants, injectables, combined oral contraceptives, some intrauterine contraceptive devices, sexual abstinence or a vasectomized partner)
- Patients requiring treatments with non-permitted medication (i.e. 5-HT3 antagonist, spasmolytics, anticholinergics, cholestyramine, anti-flatulence agents, metoclopramide, gastric-anti secretory agents (proton pump inhibitors; for indications other than Gastroesophageal Reflux Disease (GERD)), narcotics, anti-diarrheal drugs, and systemic steroids)
- Patients requiring the use of antibiotics either in medicine form of natural (e.g. grapefruit seed extract, olive leaf extract, oil of oregano, colloidal silver and highly concentrated garlic preparations)
- Exercise and the use of complementary and alternative medicine for IBS symptoms (i.e. peppermint oil, cognitive behavior therapy) during the study should be maintained at the same level prior to the study.
- Patients exceeding the treatment limits of permitted medication [(more than 2 days/week during the study period): alginate, antacids and analgesics (limited to acetaminophen ≤ 1000 mg/day, acetylsalicylic acid or NSAIDS no more than 2 tablets/day), (stable dose throughout the study period, anti-depressants (must be on a stable dose > 3 months), fiber supplements, psyllium hydrophilic mucilloid, gastric anti secretory agents (only for GERD patients who are on a stable dose > 3 months; patients should be able to differentiate between IBS and GERD symptoms), acetylsalicylic acid ≤ 325 mg/day, sedatives. Deliverance medications: Mild laxatives only if necessary.]. Any other medications can be used without limits based on the clinical judgment of the treating investigator.
- Being in another clinical trial 4 weeks before entering the study
- Constipated IBS patients
- IBS-Diarrhea patients with un-treated lactose intolerance
- Regular use of probiotics or using other probiotics during the course of the study
- Patients allergic to milk or soy products
- Patients using catheters
- Patients presented with rectal bleeding, weight loss, iron deficiency anemia, nocturnal symptoms and a family history of colorectal cancer, inflammatory bowel disease and celiac spruce

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- Patients over 50 diagnosed with Irritable Bowel Syndrome who have not had a colonoscopy in the last 5 years
- Patients who have allergies for the active ingredients or any of the excipients
- Patients presented with any immune-compromised condition (such as AIDS, lymphoma, long term corticosteroid treatment)
- Patients presented with nausea, vomiting and fever

Patient withdrawal from the study:

All patients are free to withdraw from the study at any time with or without providing a reason for their withdrawal.

Drop-outs are the patients who decide to leave the study before the planned finishing time for whatever reason. Withdrawals are the drop-outs who are withdrawn from the study for the following reasons:

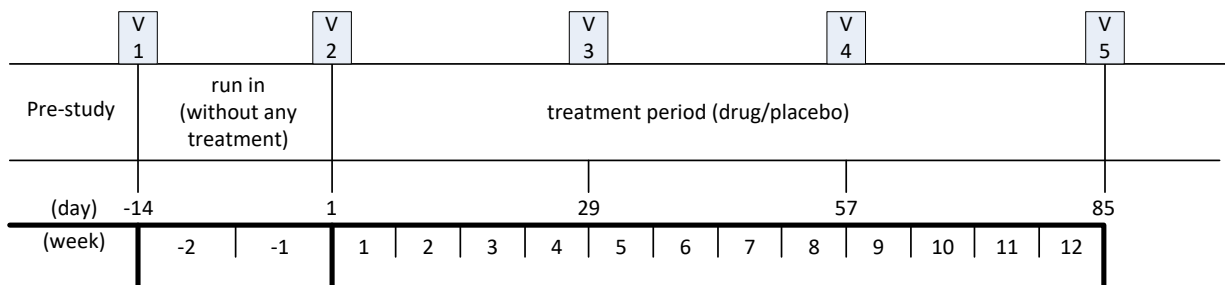
- Cannot tolerate the adverse events
- Lack of efficacy
- Insufficient compliance
- Protocol violation

Since the analysis will be conducted on 'Intention to Treat' basis, the last available data from the drop-outs will be carried forward in the analysis.

Screening and Recruitment:

Figure 1 below describes the overall study design:

Figure 1: Overall Study Design



V = patients visit

The study will be seeking to recruit IBS patients via advertising through local radio stations, local news papers and flyers.

Visit 1: 'Run- In' And Recruitment visit. Week -2:

Interested potential participants will be asked to contact NACTRAC where they will undergo a thorough assessment of their fitness for inclusion into the study including determination of their status as an IBS-Diarrhea patient using the following criteria:

- IBS-Diarrhea patient:
 - IBS definition will be based on Rome criteria;
The symptoms of IBS must persist for at least 3 months and must include:
 1. Abdominal pain or discomfort which is relieved by defecation, and/or associated with a change in frequency of stool and/or consistency of stool
 2. At least two of the following, at least a quarter of occasions or days (25%):
 - a. Altered stool frequency (≥ 3 bowel movements/day or < 3 bowel movements/week)
 - b. Altered stool form (lumpy/hard or loose/watery stools)
 - c. Altered stool passage (straining, urgency or feeling of incomplete evacuation)
 - d. Passage of mucus
 - e. Bloating or feeling of abdominal distention
 - Diarrhea is defined as having loose watery stools at least three times per day

The potential participants will also undergo the following assessments/procedure:

- Informed consent
- Medical history
- Demographic data
- Physical examination
- Upper GI symptoms
- Relationship between bowel motions and IBS symptoms
- Baseline complaints
- Concomitant medications
- Pregnancy test

Patients who meet the inclusion criteria will be asked to come back to NACTRC after 14 days. These 14 days are going to be used as 'run-in' period where patients are going to undergo the following:

- Asked to stop the non-permitted medications immediately.
- Asked to follow the following dietary recommendations:
 1. Eliminate or greatly reduce sugars from their diet. This includes refined sugar, brown sugar, syrups, molasses, agave, cane juice and honey. As

sugars play a major role in the overgrowth of small intestinal bacterial and other pathogens such as fungi.

2. Avoid known trigger foods, such as fatty fried foods and spicy foods, and additionally any foods that the patient has a known allergy or intolerance to, such as tomatoes, eggs, etc.
 3. Eat processed foods only in moderation
- No study treatment will be given
 - Diaries will be issued for each patient with a detailed explanation on its use. Diaries will include the overall subjective assessment of the following (based on the recommendations of the Rome committee on treatment trials (7)):
 1. Stool frequency (number of stools/day)
 2. Abdominal pain [on a scale of 0-4 (0 is none, 1 mild, 2 moderate, 3 severe and 4 is incapacitating)]
 3. Stool consistency [on a scale of 1-7 according to the Bristol stool form scale (1 is separate hard lumps, 2 is sausage shaped but lumpy, 3 is like a sausage or a snake but with cracks on the surface, 4 is like a sausage or a snake, smooth and soft, 5 is soft blobs with clear-cut edges, 6 is fluffy pieces with ragged edges, a mushy stool and 7 is watery, no solid pieces)]
 4. Straining (yes (1) or no (0) question)
 5. Urgency (yes (1) or no (0) question)
 6. Feeling of incomplete defecation (yes (1) or no (0) question)
 7. Bloating (yes (1) or no (0) question)
 8. Passage of mucus (yes (1) or no (0) question)
 9. Any medication taken for bowel movement (either for diarrhea or constipation)
 10. Food frequency and kind to assess the ingestion of potential symptom-exacerbating foods

Visit 2: Baseline visit. Week 0:

At baseline visit patients will be excluded if:

- The patient developed any of the exclusion criteria mentioned earlier
- The patient did not complete the diary adequately
- The patient rated his/her abdominal pain as incapacitating at least twice during the 'run-in' period
- The patient reported not having abdominal pain/discomfort for more than 50% of the 'run-in' period
- The patient did not have a bowel movement for more than 4 days (either consecutive or non-consecutive) during the 'run-in' period

Excluded patients will be replaced within the study. If the patient is fit to be in the study he/she will be randomized into either of the study groups on the following basis:

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The patients are going to be randomized (on 1:1 basis) via a centralized secure website to ensure allocation concealment into either the 'intervention' group or the 'placebo' group. Both the patients and the treating investigators are going to be blinded to the treatment.

Patients in the 'intervention' group will receive PXO612 which is a probiotic composed of the following ingredients contained in a veggie capsule, being one dose:

Bacillus coagulans	200 million colony forming units	33.34mg
Bacillus subtilis	100 million colony forming units	16.67mg
Enterococcus faecium	100 million colony forming units	16.67mg
Fructo-oligosacharride	a nutrient for the packaged product	600.0mg
Total		668.68 mg

Bacillus coagulans is a non-pathogenic, Gram positive, spore forming bacteria that produces lactic acid. Though not normally found in the gut. Bacillus coagulans strains have been used as general nutritional supplements and agents to control constipation and diarrhea in humans and animals.

Bacillus subtilis is a Gram-positive, endospore-forming soil bacteria comprising aerobic and a few facultatively anaerobic rod shaped bacteria. Bacillus subtilis was historically used to treat dysentery.

Enterococcus faecium is a facultative (growing with or without oxygen) anaerobic, Gram positive cocci that produces lactic acid. The Enterococcus faecium strain is a natural inhabitant of the mammalian GI tract.

Patients in the 'placebo' group will receive the placebo capsules. The main ingredient in the placebo capsule is Di-Calcium Phosphate

After the randomization the treating investigator will review the patient's diary and the patients will undergo the following:

- Bowel movements (stool frequency) will be assessed at each visit using the patient's reported stool frequency/day
- The overall symptoms relief will be calculated at each visit by summing the following reported symptoms scores (based on the recommendations of the Rome committee on treatment trials (7)):
 - Stool frequency (number of stools/day)
 - Abdominal pain (on a scale of 0-4)
 - Stool consistency (on a scale of 1-7)

- Straining (yes (1) or no (0))
 - Urgency (yes (1) or no (0))
 - Feeling of incomplete defecation (yes (1) or no (0))
 - Bloating (yes (1) or no (0))
 - Passage of mucus (yes (1) or no (0))
 - Subjective assessment of the upper GI symptoms [frequency of vomiting per week, heartburn, early satiety, postprandial fullness, sensation of prolonged digestion and nausea on a scale of 0-4 (0 is none, 1 mild, 2 moderate, 3 severe and 4 is incapacitating)]
 - Quality of life assessment using SF-36
 - Any medication being taken by the patient
- At the end of each visit the patients will be provided with the drug or placebo supply that would be enough for them until the next visit.
- Patients will be asked to keep filling their diary as requested over the study period; as their diary will be reviewed at each visit by the treating investigator.

Visits 3, 4 and 5. Weeks 4, 8 and 12:

If the patient developed any of the exclusion criteria during the course of the study he/she will be withdrawn from the study

Bowel movements (stool frequency) will be assessed at each visit (3, 4 and 5) using the patient's reported stool frequency/day.

The overall symptoms relief will be calculated at each visit (3, 4 and 5) by summing the following reported symptoms scores (based on the Rome criteria):

1. Stool frequency (number of stools/day)
2. Abdominal pain (on a scale of 0-4)
3. Stool consistency (on a scale of 1-7)
4. Straining (yes (1) or no (0))
5. Urgency (yes (1) or no (0))
6. Feeling of incomplete defecation (yes (1) or no (0))
7. Bloating (yes (1) or no (0))
8. Passage of mucus (yes (1) or no (0))

The following subjective information is going to be collected at visits 3 and 4:

- Subjective assessment of upper GI symptoms [frequency of vomiting per week, heartburn, early satiety, postprandial fullness, sensation of prolonged digestion and nausea on a scale of 0-4 (0 is none, 1 mild, 2 moderate, 3 severe and 4 is incapacitating)]
- Compliance with the treatment (using pill count)

- Adverse events
- Any non-study medication

At visit 5 the patient will undergo the following:

- Compliance assessment (using pill count)
- Subjective assessment of the upper GI symptoms [frequency of vomiting per week, heartburn, early satiety, postprandial fullness, sensation of prolonged digestion and nausea on a scale of 0-4 (0 is none, 1 mild, 2 moderate, 3 severe and 4 is incapacitating)]
- Quality of life assessment using SF-36
- A Functional Bowel Disorder Severity Index (FBDSI) evaluation
- Adverse events assessment
- Any non-study medication

Dosing regimen:

The patients will start taking 1 capsule per day with any meal. At the beginning of week 3, they will take one capsule with breakfast and one with their evening meal. At beginning of week 7, they will take one in morning, one at lunch and one at the evening meal. The patients will be asked to keep their sugar intake to a minimum.

Outcomes:

Primary outcome:

The primary outcome is the difference in change in the bowel movements (stool frequency) between the 'intervention' group and the 'placebo' group over the study period. **Secondary outcomes:**

- Difference in the overall symptoms relief between the 'intervention' group and the 'placebo' group over the study period
- Differences in abdominal pain/discomfort, stool consistency, stool frequency, straining, urgency, bloating, feeling of incomplete defecation and passage of mucus between the 'intervention' group and the 'placebo' group over the study period
- Differences in upper GI symptoms (i.e. heartburn, early satiety, postprandial fullness, sensation of prolonged digestion, nausea and vomiting) between the 'intervention' group and the 'placebo' group over the study period
- Differences in quality of life using SF-36 between the 'intervention' group and the 'placebo' group over the study period
- Changes in severity of IBS symptoms as determined by pre- and post-study FBDSI scores

Sample Size:

Using the information from Dolin (2009) (approximate reduction of 0.47 in the bowel movements in the placebo group versus 0.8 in the treatment group), power of 80% and

95% confidence interval. 78 patients (39 in each group) are required to be able to detect a minimum of 0.33 difference in change in the bowel movements between the 'intervention' group and the 'placebo' group over the study period.

Statistical Analyses:

Interim Analysis

An interim analysis will be conducted after a minimum of 20 patients in each study group have completed the trial. The interim analysis will include all safety and efficacy data. For the interim analysis, the study data will be unblinded to the biostatistician or sponsor representative. Trial success will be demonstrated if the change in bowel movements is statistically different at an alpha of 0.01. If it is not statistically significant at the interim, then recruitment will proceed as planned.

All analysis will be conducted on 'Intention to Treat' basis. A comparison of baseline characteristics will be performed using t-tests or nonparametric Wilcoxon for continuous variables and chi-squared test for categorical variables. In order to account for any imbalance in baseline characteristics, test for differences in change in bowel movements between the 'intervention' group and the 'placebo' group will be adjusted for using multiple regression models (or analysis of covariance models). The problem of missing values will be checked for non-randomness and appropriate imputation methods will be applied when needed.

Adverse events:

Serious adverse event (SAE) or serious adverse reaction: any untoward medical occurrence that occur at any dose:

- Results in death,
- Is life threatening, (Note: the term life-threatening refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/ reaction which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or results in prolongation of existing hospitalization
- Results in persistent or significant disability/ incapacity
- Is a congenital anomaly/birth defect
- Is a medically important event or reaction. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalization, but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed in the definition above.

Related SAE: there is a reasonable possibility according to the treating investigator that the Product may have caused the event.

Unusual lack of efficacy: When a health product fails to produce the expected intended therapeutic effect.

Adverse events will be collected and reported to the appropriate authorities through the appropriate channels.

Patients will be withdrawn from the study if they suffered from any serious adverse events.

References:

1. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. *Gastroenterology*. 2006; 130:1480-1491.
2. Longstreth GF, Wilson A, Knight K, et al. Irritable bowel syndrome, health care, and costs: a U.S. managed care perspective. *Am J Gastroenterol*. 2003; 98: 600-607.
3. Wilson A, Longstreth GF, Knight K, et al: Quality of life in managed care patients with irritable bowel syndrome. *Manage Care Interface*. 2004; 17: 24-28.
4. Canadian Digestive Health Foundation. Irritable Bowel Syndrome; 2011. Available: http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf (accessed Jan 2012).
5. Drossman DA. Review article: An integrated approach to the irritable bowel syndrome. *Aliment Pharmacol Ther*. 1999; 13: (Suppl 2): 3-14.
6. Pharmabiotix INC. Investigator brochure. 2011, 1-15.
7. Schoenfeld p, Talley NJ. Measuring successful treatment of irritable bowel syndrome: is satisfactory relief enough? *Am J Gastroenterol*. 2006; 101: 1066-1068.
8. Dolin BJ. Effects of a proprietary *Bacillus Coagulans* preparation on symptoms of diarrhea-predominant irritable bowel syndrome. *Methods Find Exp Clin Pharmacol*. 2009; 31: 655-659